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An emerging role for NAADP-mediated Ca^{2+} signaling in the pancreatic β-cell

Abdelilah Arredouani,[1] A. Mark Evans,[2] Jianjie Ma,[3] John Parrington,[1] Michael X. Zhu[4] and Antony Galione[1,*]

[1]Department of Pharmacology; University of Oxford; Oxford, UK; [2]Centre for Integrative Physiology; College of Medicine and Veterinary Medicine; University of Edinburgh; Edinburgh, Scotland UK; [3]Department of Physiology and Biophysics; UMDNJ-Robert Wood Johnson Medical School; Piscataway, NJ USA; [4]Department of Integrative Biology and Pharmacology; The University of Texas Health Science Center in Houston; Houston, TX USA

Several recent reports, including one in this journal, have reignited the debate about whether the calcium-mobilizing messenger, nicotinic adenine nucleotide diphosphate (NAADP) plays a central role in the regulation of calcium signaling in pancreatic β-cells.[1-5] These studies have highlighted a role for NAADP-induced Ca^{2+} mobilization not only in mediating the effects of the incretin, GLP-1 and the autocrine proliferative effects of insulin, but also possibly a fundamental role in glucose-mediated insulin secretion in the pancreatic β-cell.

Key words: pancreatic β-cell, calcium lysosome, two-pore channel (TPC), NAADP

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*Correspondence to: Antony Galione; Email: antony.galione@pharm.ox.ac.uk

Introduction

The K_{ATP} channel-dependent hypothesis for stimulus-secretion coupling in pancreatic β-cells has gained prominence in recent years as the major pathway by which glucose triggers signaling events to bring about membrane depolarization and the activation of voltage-dependent calcium channels (VDCCs) and Ca^{2+} influx, the major regulator of insulin granule exocytosis.[6] However, several shortcomings of this hypothesis have been articulated.[7,8] First, glucose may still evoke Ca^{2+} signals and insulin secretion after knockout of K_{ATP} channel components,[9-11] and secondly K_{ATP} channel closure alone is not sufficient to depolarize the membrane to cause activation of VDCCs. A background current, for example, an inward cation current is required, together with the closure of the K_{ATP} channel which itself increases the membrane resistance to cause sufficient depolarization to activate VDCCs. The Ca^{2+} mobilizing messenger, NAADP is gaining prominence as a universal trigger for events at both the ER and at the plasma membrane.[12-15] It does this by causing local Ca^{2+} release from acidic stores, which may then be amplified by recruiting Ca^{2+} release channels on the endoplasmic reticulum (ER) membrane or activating Ca^{2+}-dependent channels at the plasma membrane. Recent studies have demonstrated the presence of NAADP-sensitive acidic Ca^{2+} stores in pancreatic β-cells,[1,16-18] and their role in triggering signaling events in β-cells is gathering momentum.

The most recent reports highlight the role of NAADP in signal transduction in response to activation of autocrine insulin receptors.[4,5] Another highlights the role of NAADP in mediating the effects of the incretin, GLP-1.[1] Furthermore, a recent important development describes a new selective membrane-permeant NAADP antagonist, Ned-19 which inhibited glucose-evoked calcium spiking in mouse pancreatic β-cells in a concentration-dependent manner.[2] Finally, a groundbreaking discovery has been made recently with the identification of the two pore channels (TPCs) as NAADP-gated calcium release channels on endo-lysosomal membranes and the demonstration that NAADP evoked oscillatory non-selective cation currents in mouse pancreatic β-cells, this effect being abolished in cells prepared from *Tpc2*$^{-/-}$ mice.[3] These studies represent the re-emergence of an interest in NAADP signaling in pancreatic β-cells that occurred some years ago and cement earlier observations on a possible role for

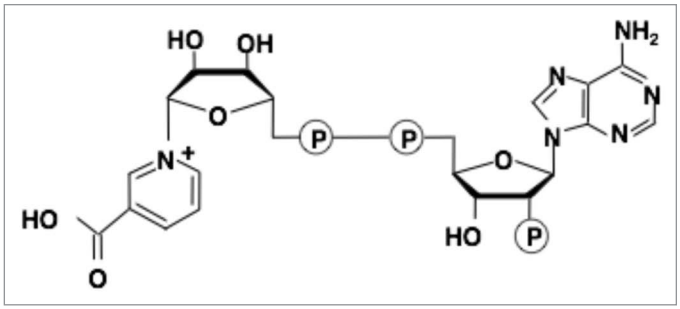

Figure 1. Structure of NAADP. NAADP differs from β-NADP only in that that the base nicotinic acid is substituted for nicotinamide.

NAADP in glucose-induced calcium signaling in these cells.[19] These earlier studies suggested a role for NAADP in mediating the autocrine effects of insulin in human islets,[20] the release of Ca^{2+} from acidic stores in β-cell lines,[16] and the role of NAADP-evoked calcium release as a critical mediator of Ca^{2+} signaling responses to glucose in MIN6 cells.[17,21] With these new studies, NAADP is emerging as an important player in the regulation of β-cell Ca^{2+} signaling.

NAADP as a Calcium Mobilizing Messenger

NAADP is the most potent of the major Ca^{2+} mobilizing messengers described, with activity reported at low nanomolar intracellular concentrations.[22] It differs in structure from the more familiar coenzyme NADP by the substitution of the nicotinamide moiety by nicotinic acid (**Fig. 1**). Its actions were first reported in sea urchin eggs,[22] but NAADP is now known to have widespread actions in most cell types studied.[13,23] However, it is fair to say that in terms of its synthesis, regulation and mechanism of action, NAADP signaling is poorly understood. NAADP is thought to be synthesized from NADP as a precursor, and the only mammalian enzymes that have been demonstrated to catalyze this reaction, at least in vitro, are ADP-ribosyl cyclases (ARCs) such as CD38.[24,25] Interestingly, CD38 and its alternate Ca^{2+} mobilizing product, cADPR, had previously been linked to stimulus-secretion coupling in β-cells[26,27] as discussed below. Various stimuli, including agonists acting at a variety of cell surface receptors, have been demonstrated to increase cellular NAADP levels, adding to growing evidence that it is an intracellular messenger.[23] The concentration-response relationship between NAADP and Ca^{2+} release in β-cells,[20,21] in common with other mammalian cell types, is described as bell-shaped, with maximal Ca^{2+} release evoked by around 100 nM NAADP.[28] Higher concentrations are less effective; with micromolar concentrations causing apparent desensitization of the NAADP-sensitive Ca^{2+} release mechanism in the absence of measurable Ca^{2+} release.[1,20,21] In many cell types, NAADP appears to target a separate Ca^{2+} store, which may be acidic in nature such as lysosomes, rather than the long-established ER Ca^{2+} store,[29] although Ca^{2+} release from acidic stores can trigger subsequent Ca^{2+}-induced Ca^{2+} release (CICR) from the ER.[14,30,31] Thus NAADP has been proposed to serve as a key triggering Ca^{2+} mobilizing messenger, not only releasing Ca^{2+} by directly targeting NAADP-sensitive stores, but also acting to coordinate Ca^{2+} release from the ER via IP_3R and RyR through CICR-dependent mechanisms.[12,14]

The Molecular Identity of NAADP Receptors

The molecular targets for NAADP have been a matter of debate for some time.[32] Pharmacological studies have generally supported the idea that NAADP targets a mechanism distinct from the two major Ca^{2+} release channels, IP_3 or RyRs. Of the known lysosome resident channels, mucolipin 1 was the first to be suggested as the target receptor channel for NAADP[33] but this protein had previously been ruled out by others.[34] Ryanodine receptors have also been suggested in some cells to be directly targeted by NAADP,[35,36] although this is not universally accepted.[37] However, the recent identification of two-pore channels (TPCs) as NAADP receptors,[3] this finding being subsequently confirmed by others,[38,39] perhaps provides the most convincing candidates, as they mirror many of the properties previously ascribed to NAADP-gated Ca^{2+} release mechanisms including endo-lysosomal localization.[14,40]

TPCs are poorly characterized members of the voltage-gated superfamily of cation channels. They are an ancient family, and a TPC homolog functions as a vacuolar channel in plants.[41] They consist of two-linked 6 transmembrane domain units and thus have a total of 12 transmembrane alpha helices. It is likely that the functional channel is a dimer. There are three non-allelic TPC genes in most animal species, but only TPC1 and TPC2 are found in humans and rodents. Expression of HsTPCs in HEK293 cells showed them to be localized to the endolysosomal system, with TPC2 predominantly expressed in lysosomes while TPC1 was endosomal. In cells overexpressing TPC2, NAADP evoked a biphasic Ca^{2+} release, the first phase from acidic stores, whilst the second phase was due to amplification by the recruitment of ER IP_3Rs, presumably by CICR.[3,40] The concentration-dependence of NAADP action showed the characteristic bell-shaped concentration-response curve. siRNA TPC2 probes abolished the sensitivity to NAADP, and stimulation of Ca^{2+} activated cation currents in mouse pancreatic β-cells were absent in cells from *Tpc2*$^{-/-}$ mice. In addition, expression of TPC2 in HEK293 cells was associated with increased [^{32}P]NAADP binding,[3] and immunopurified endogenous sea urchin TPC1 and TPC3 complexes bind [^{32}P]NAADP with nanomolar affinity.[40]

Taken together, TPCs represent strong candidates as NAADP receptors[14,15,42] and provide for the first time a molecular handle with which to investigate NAADP signaling.

Calcium Stores in Pancreatic β-cells

The traditional model for glucose-evoked Ca^{2+} signaling has emphasized the role of

Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels, with a minor role for the ER in shaping the stereotypic Ca^{2+} spikes. Indeed, the earliest Ca^{2+} response observed to be initiated by glucose is a decrease ascribed to enhancement of Ca^{2+} uptake by the ER by increase in ATP synthesis[43] probably mediated by SERCA2b. Pharmacological ablation of SERCA-mediated pumping of Ca^{2+} into the ER with thapsigargin or genetic ablation of SERCA2b and 3 isoforms in mouse β-cells generally enhances amplitudes of glucose-evoked Ca^{2+} oscillations.[44] Thus the ER plays a role in Ca^{2+} buffering, although cycles of uptake and release may be important in glucose-mediated fast Ca^{2+} spiking[45] and play a prominent role in the modulation of Ca^{2+} spiking by activation of incretin receptors coupled to IP_3 production.[46] The finding that NAADP targets acidic Ca^{2+} stores[29] has prompted the investigation of the role of acidic stores in Ca^{2+} handling in pancreatic β-cells.

In contrast to the ER, little is known of the Ca^{2+} uptake mechanisms in acidic stores, nor how Ca^{2+} is stored in these organelles, although polyanions have been suggested to play a role.[47] However, Ca^{2+} uptake and storage seem to be dependent on the maintenance of the pH gradient across their membranes. Two classes of pharmacological agents have been principally used to abrogate Ca^{2+} storage by these organelles. The first is bafilomycin and related compounds such as concanamycin, which inhibit the vacuolar H^+-ATPase expressed widely in acidic organelles including the endolysosomal system and secretory granules.[48] The ability of these compounds to discharge Ca^{2+} depends on the leakiness of stores to both protons and Ca^{2+} and often requires prolonged incubations. Both nigericin, a protonophore and NAADP have been found to accelerate Ca^{2+} leak by these organelles.[29] A second compound, glycylphenylalanine-2-naphthylamide (GPN), causes osmotic lysis of acidic stores based on the presence of cathepsin C, found principally within lysosomes, and is accompanied by bursts of Ca^{2+} release.[29] In MIN6 cells in which the bioluminescent Ca^{2+} reporter aequorin was targeted to the ER or insulin-containing secretory granules, it was found that NAADP released Ca^{2+} from secretory granules but not the ER, an effect that was not blocked by either ryanodine or dantrolene, a RyR1 inhibitor.[16] It was found that photolysis of caged NAADP evoked a Ca^{2+} response that was largely abolished by pretreatment with bafilomycin, but thapsigargin did not affect the amplitude, although it reduced the duration of the response.[17] A further study showed in mouse primary β-cells that NAADP-evoked Ca^{2+} release was blocked by GPN and partially inhibited by thapsigargin but not by dantrolene.[18] Acidic Ca^{2+} pools were shown to be primarily responsible for the delayed plateau of Ca^{2+} release after prolonged depolarization of β-cells termed the "Ca^{2+} hump". The apparent large size of this Ca^{2+} pool may indicate that Ca^{2+} release from the small number of lysosomes in β-cells alone cannot account for this phenomenon, but might include secretory granules or other acidic organelles in addition.[18] Additionally, in one of the recent studies, NAADP was firmly shown to mobilize Ca^{2+} from acidic stores but not the ER in mouse primary β-cells.[1] However, in permeabilized mouse β-cells, NAADP was found to be ineffective at mobilizing Ca^{2+}.[49] There are few reports of NAADP efficacy in broken cell systems.[28] One possible explanation is that stores are labile, small and coupling to CICR amplification mechanisms required for detection are broken.

NAADP Synthesis and Regulation

An impetus for studying the role of NAADP in β-cells followed on from the work of Okamoto and colleagues. In 1993, they proposed a key role for cADPR in stimulus-secretion coupling.[26] cADPR was proposed to enhance insulin secretion in pancreatic β-cells by either mobilizing Ca^{2+} from the ER through activating RyRs[26] or at higher concentrations by enhancing Ca^{2+} influx through activating plasma membrane TRPM2 channels.[50] Inhibition or genetic ablation of CD38 function has been correlated with reduced Ca^{2+} responses and insulin secretion in response to elevated glucose concentrations,[51] and even linked to Type 2 diabetes.[27,52-55] Subsequent studies showed that CD38, which cyclizes NAD to produce cADPR, played a key role in insulin secretion.[51] However, given that cADPR targets RyRs mainly on the ER, and this organelle shapes, rather than has a triggering role in evoking Ca^{2+} signals in β-cells,[56] and that a role for cADPR in β-cell stimulus-secretion coupling has not been universally confirmed,[57,58] the role of NAADP was investigated since it was shown that CD38 and ARCs could catalyze NAADP formation in cell-free systems[24] and NAADP mobilizes Ca^{2+} largely from a store separate from the ER.

While a number of studies have provided evidence for the role of ARCs such as CD38 in the synthesis of cADPR,[59] evidence for their role in the synthesis of endogenous NAADP remains conflicting, with some studies supporting such a role,[60,61] others arguing against.[62] In a recent study in β-cells, CD38 was shown to contribute to NAADP synthesis stimulated by GLP-1, but this appeared not to be the only mechanism for generating NAADP.[1]

CD38 was initially considered an ectoenzyme[59] but it has now been shown that ARCs may also be localized inside cells, where they are more appropriately sited for production of intracellular messengers.[63] There is growing evidence that ARCs, including CD38, may be present in endosomes,[64] secretory granules[63] and even lysosomes.[1] In an interesting study, internalization of plasma membrane CD38 was induced by the reducing agent, L-oxothiazolidine-4-carboxylic acid, a pro-drug of cysteine, which like glucose enhances dimerization of CD38, enhances glucose-mediated Ca^{2+} signaling and induces antidiabetic effects in ameliorating glucose intolerance in db/db mice.[64] The localization of messenger-synthesising enzymes within organelles presents a topological problem in that substrates have to be transported in, and products out to their targets. However, evidence has been presented in the sea urchin egg for such processes.[63] Moreover, the luminal acidic pH may favour enzyme activities especially for NAADP generation and since a base-exchange mechanism has been proposed for this messenger,[24,25] nicotinic acid may accumulate there at sufficient concentrations for NAADP synthesis by this mechanism.

Although NAADP has been found to increase in islets and β-cells in response to glucose, GLP-1 and insulin, stimulus-coupling to intra-organellar enzymes again presents a topological problem. At present these mechanisms are unclear. However, in β-cells, ATP,[65] cGMP-dependent kinases, PKC[4] and cAMP (perhaps via EPAC)[1] have all been proposed. Our understanding of the way in which β-cell agonists couple to the enzymatic synthesis of NAADP and cADPR is in urgent need of further study in common with the situation in other systems, as is the identification of other possible NAADP synthases.

NAADP and Receptor-mediated Ca^{2+} Signaling

Incretins such as GLP-1 do not trigger insulin secretion per se but potentiate the actions of glucose.[66] They act at cell surface receptors and activate signal transduction processes. A recent report has demonstrated that GLP-1 is coupled to both NAADP and cADPR synthesis and Ca^{2+} release from acidic and ER stores.[1] Unusually in this study, NAADP was shown to increase Ca^{2+} in β-cells in response to extracellular application of NAADP at concentrations similar to those required for responses in studies employing whole cell patch clamp application.[2]

GLP1 (10 nM) was found to evoke Ca^{2+} signals only in the presence of elevated glucose.[1,67] Ca^{2+} signals were maintained with the initial phase ascribed to NAADP whilst thapsigargin and cADPR antagonists (but not IP_3R blockers) caused the abolition or abbreviation of the second maintained phase. Both desensitizing concentrations of NAADP or pretreatment with bafilomycin abolished both phases of the GLP-1 response. Thus the initial transient evoked by NAADP is required for the second phase of Ca^{2+} release evoked by cADPR. This is similar to models of NAADP trigger action in other systems[12] such as the fertilization of the sea urchin egg, where NAADP can fire a Ca^{2+} wave first by evoking Ca^{2+} release from acidic stores and then by recruiting ER CICR mechanisms.[68,69]

Experiments with *Cd38*$^{-/-}$ islets showed a partial dependence on CD38 for both NAADP and cADPR synthesis, suggesting other enzymes may also be involved in their production.[1]

Another receptor on β-cells linked to Ca^{2+} signals dependent on NAADP-sensitive Ca^{2+} release is the insulin receptor. Paracrine or autocrine effects of insulin receptors have been shown to evoke Ca^{2+} signals in β-cells.[70,71] In the first report of NAADP effects in human β-cells, microinjection of NAADP at different concentrations displayed the hallmark bell-shaped curve for Ca^{2+} release with maximum effects at 100 nM. Injecting a desensitizing concentration of 10 mM, insulin-evoked Ca^{2+} signals were abolished. Furthermore, the insulin concentration-response curve was bell-shaped for Ca^{2+} release, mirroring that for NAADP. As for GLP-1, the initial phase of Ca^{2+} release evoked by insulin was insensitive to SERCA pump inhibitors suggesting release from a non-ER store. The second phase of insulin-evoked Ca^{2+} release was partially blocked by the IP_3R antagonist, xestospongin but not by ryanodine. Insulin stimulation of islets lead to increased insulin content but not secretion, thus implicating a role for NAADP-mediated Ca^{2+} signaling in insulin regulation of insulin gene expression. Interestingly, as we describe below, while NAADP may depolarize β-cell membranes (**Fig. 2**), insulin apparently does not.[70]

As recently reported in this journal, the role of NAADP in the mediation of insulin responses has been reinvestigated. In this study, NAADP was again implicated in initiating the Ca^{2+} mobilizing effects of insulin, with a sequential role for IP_3 and cADPR consistent with the triggering role for NAADP. Here a role for insulin-mediated NAADP-dependent signaling was implicated in the control of β-cell proliferation.[4] Additionally, insulin was shown to increase islet NAADP levels, in contrast to the previous report from the same group.[1]

A Triggering Role for NAADP in Nutrient-Mediated Calcium Signaling

Perhaps one of the most interesting new aspects in this area is that a key role for NAADP for the actual triggering of glucose-mediated responses has now been reported in several studies. The first study to suggest this role used the ability of high concentrations of NAADP to desensitize NAADP-evoked Ca^{2+} release[21] in MIN6 cells. Using microinjected caged NAADP it was found that photolysis of low amounts evoked a large Ca^{2+} release, which was largely resistant to inhibition by thapsigargin. However, with photolysis of higher amounts of caged NAADP, no Ca^{2+} release was seen. Having established conditions for desensitization of NAADP-evoked Ca^{2+} release, cells were then challenged with 20 mM glucose. Glucose-evoked Ca^{2+} oscillations were greatly suppressed, suggesting a role of NAADP in the actual triggering processes in β-cell stimulus-secretion coupling. In addition, high affinity binding sites for NAADP were found in islets, and importantly glucose was able to evoke an increase in cellular NAADP levels.[21] Since NAADP was reported to mobilize Ca^{2+} from acidic stores,[29] the effects of disrupting Ca^{2+} storage in acidic organelles upon glucose-evoked Ca^{2+} signaling was examined.[17] Bafilomycin (2 μM) abolished glucose responses but not those to acetylcholine, an incretin acting on β-cell muscarinic receptors which are linked to IP_3 production and release of Ca^{2+} from ER stores. Conversely, thapsigargin abolished acetylcholine but not glucose responses.[17] Taken together, these data are suggestive of a role for NAADP-mobilization from acidic stores in the glucose-mediated triggering response. The employment of a newly developed membrane-permeant selective NAADP antagonist, Ned-19 has confirmed these findings. Ned-19 is weakly fluorescent and labels acidic stores in pancreatic β cells, an effect that is reduced by prior treatment with the membrane-permeant NAADP analogue, NAADP-AM.[2] Ned-19 abolished glucose-evoked Ca^{2+} responses in a concentration-dependent manner (**Fig. 3**),[2] although it did not affect activation of voltage-gated Ca^{2+} channels by potassium depolarization or mitochondrial metabolism. Since the large glucose-evoked Ca^{2+} signals are dependent on extracellular Ca^{2+} as they rely on depolarization-evoked openings of VDCCs, what then is the role of NAADP-mediated Ca^{2+} release from acidic stores?

One clue has come from intracellular perfusion of β-cells with NAADP. NAADP evokes a series of oscillatory depolarizing cation currents, which are abolished by Ned-19.[2] The channels carrying these currents are unknown but intriguingly they are blocked by TRPM4/5 blockers (unpublished observations), and Ca^{2+}-activated TRPM4 and TRPM5 channels in the plasma membrane have been suggested to play an important role in shaping Ca^{2+} signals[72] and controlling insulin secretion in β-cells.[73-75] Thus NAADP-evoked Ca^{2+} release via TPCs from acidic stores just under the plasma membrane could comprise a signaling module with Ca^{2+}-activated TRPM4/5 channels mediating plasma membrane depolarization by NAADP (**Fig. 4**). Interestingly, this depolarizing effect seems unique to NAADP as a Ca^{2+} mobilizing messenger, since IP_3 has been shown to hyperpolarize the β-cell membrane.[76] One possibility then is that NAADP signaling mechanisms contribute together with the closure of K_{ATP} channels to the depolarization of the β-cell membrane to a threshold for activation of VDCCs (**Fig. 2**). The operation of this triggering mechanism may explain how glucose still evokes Ca^{2+} transients in β-cells from knockout mice with defects in Kir6.2 or SUR1, the components of the K_{ATP} channel. Since NAADP production and Ca^{2+} release may be localized to regions just under the plasma membrane such Ca^{2+} microdomains, as previously hypothesized,[77] may play a key role in β-cell excitability. The currents generated by this mechanism in response to glucose may be small and transient, but sufficient to depolarize the membrane especially when the membrane resistance is increased by closure of K_{ATP} channels. Thus, although this mechanism is strictly K_{ATP}-independent, it may synergize with K_{ATP}-dependent mechanisms in the triggering phase for glucose action. A major breakthrough in our understanding of NAADP-mediated Ca^{2+} release has come from the recent discovery of two pore channels (TPCs) as NAADP-gated Ca^{2+} release channels of endolysosomal compartments.[3,14] Importantly, as described above, in β-cells from *Tpc2*$^{-/-}$ mice, NAADP no longer evokes the oscillatory depolarizing currents. The study of glucose-mediated Ca^{2+} signaling in TPC knockout mice will be important in our understanding of the role of NAADP in β-cell Ca^{2+} signaling. Preliminary results already reported suggest that glucose-evoked Ca^{2+} spiking and membrane depolarization is abolished or greatly attenuated in *Tpc2*$^{-/-}$ β-cells.[78] Further studies of *Tpc2*$^{-/-}$, *Tpc1*$^{-/-}$ and possibly *Tpc1/Tpc2* double knockout β-cells that are ongoing may provide exciting new information about a possible new triggering pathway of

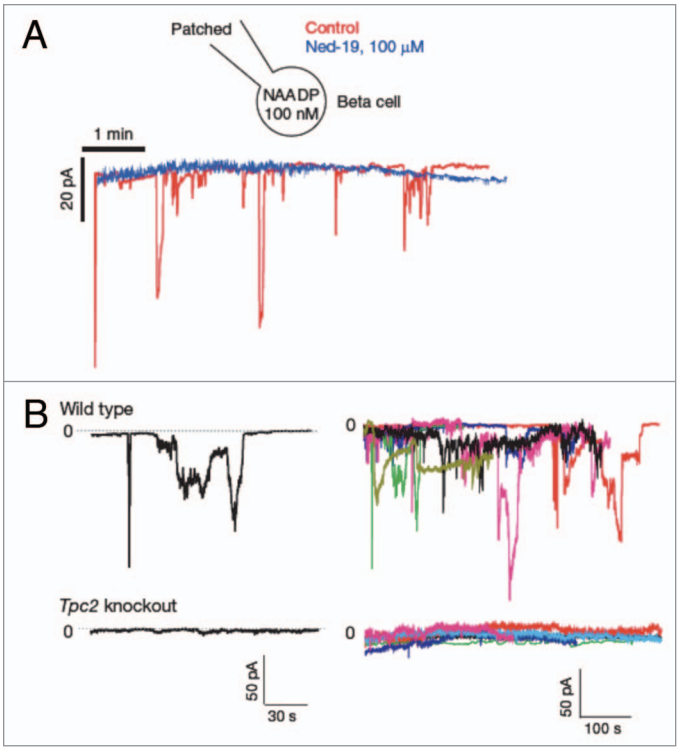

Figure 2. NAADP-evoked Ca^{2+}-activated cation currents in mouse pancreatic β-cells. (A) Ca^{2+}-dependent current traces are from mouse β cells that were voltage-clamped with a patch pipette containing 100 nM NAADP in the presence or absence of the membrane permeant NAADP antagonist Ned-19 (100 μM) in the bathing solution. (B) Cation currents at -70 mV evoked by intracellular dialysis of 100 nM NAADP via patch pipette in whole cell mode in pancreatic β-cells isolated from wild-type (top) and *Tpc2* knockout (bottom) mice. The left hand parts show a single representative trace, whilst the right hand part shows records from several cells [Taken from ref. 2 and 3].

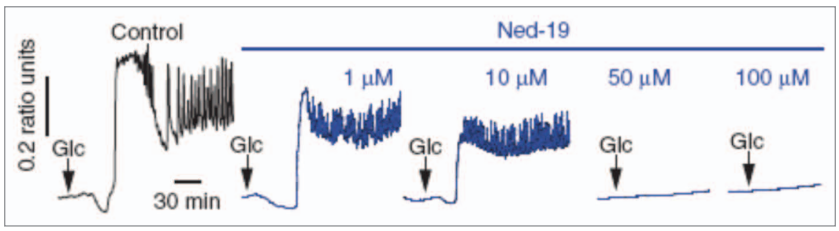

Figure 3. The NAADP antagonist blocks glucose-evoked Ca^{2+} signaling in mouse primary pancreatic beta cells. Ned-19 reduces glucose-induced Ca^{2+} increases in a concentration-dependent manner. Islets were pre-incubated with the indicated concentration of Ned-19 for 30 min before the addition of 15 mM glucose [Taken from ref. 2].

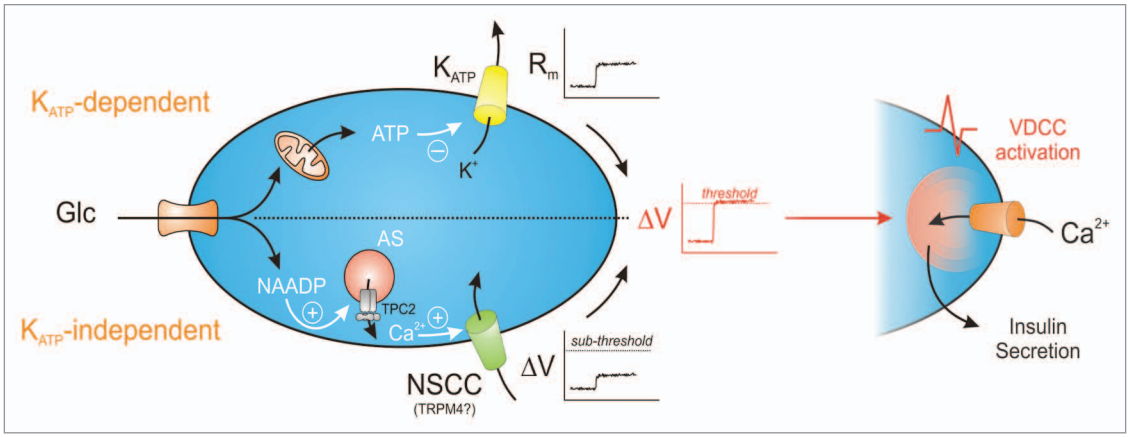

Figure 4. Scheme for how NAADP-regulated TPC2 and K_{ATP} channels synergize to trigger glucose-evoked insulin secretion. NAADP-induced Ca^{2+} release from acidic stores (AS) synergizes with the K_{ATP}-dependent pathway to depolarize the plasma membrane and activate VDCCs. The ATP-mediated closure of K_{ATP} channels increases membrane resistance, which together with Ca^{2+}-dependent depolarizing (non-selective cation) currents (possibly mediated by Ca^{2+}-activated TRPM4 or TRPM5 channels), activated by NAADP-induced Ca^{2+}-release via TPC2 expressed on acidic stores, may depolarize the plasma membrane to threshold for VDCC activation. Ca^{2+} influx via VDCCs then triggers exocytosis of insulin granules. AS: acidic stores; NSCC: non-selective cation channel; R_m: membrane resistance; VDCC: voltage-dependent Ca^{2+} channels; Glc: glucose.

NAADP for stimulus-secretion coupling in the pancreatic β-cell.

Conclusions

There are now a growing number of reports that NAADP mobilizes Ca^{2+} in pancreatic β-cells. The effects of this messenger appear to be more robust and less controversial compared to those of cADPR. The generation of selective pharmacological inhibitors for NAADP-evoked Ca^{2+} release and the identification of TPCs as target NAADP-gated Ca^{2+} release channels provide new tools for the study of NAADP-mediated Ca^{2+} signaling in pancreatic β-cells. NAADP appears to have multiple roles, which may depend on the agonist that generates its production. Whilst insulin stimulated NAADP production may control insulin synthesis and β-cell proliferation, GLP-1-mediated NAADP production may potentiate glucose-mediated Ca^{2+} signaling and insulin secretion. Perhaps most intriguing of all, NAADP-mediated Ca^{2+} release from acidic stores appears to play a key role in triggering glucose-mediated Ca^{2+} signaling. The mechanism is unclear but seems to involve the activation of calcium-activated plasma membrane cation currents which, with closure of K_{ATP} channels, may be an important component of the triggering pathway in stimulus-secretion coupling in the pancreatic β-cell. The characterization of these currents, and the elucidation of the mechanisms coupling glucose metabolism to NAADP production are urgently required.

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